Pacific Science (1986), vol. 40, nos. 1–4

ABSTRACTS OF PAPERS

Eleventh Annual Albert L. Tester Memorial Symposium 3–4 April 1986[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the University and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Jared Diamond of the University of California, Los Angeles Medical Center, participated in the symposium.

Spirulina as a Pigment and Protein Source for Poultry

DONALD W. ANDERSON[2]

The blue-green algae spirulina (*Spirulina platenesis*), which contains 55 to 65% protein, has been used as a feedstuff for poultry and other livestock species. It is also rich in xanthophylls, a group of carotenoid pigments. When consumed by poultry, these are deposited in egg yolk and skin, providing a desired yellow pigmentation. The purpose of these experiments was to determine whether the xanthophyll and protein components of spirulina could be separated, thus yielding two salable commodities.

Four feeding trials were conducted. In trial 1, freeze-dried spirulina was fed to Japanese quail at 0.25, 0.5, 1, 2, and 4%, using a xanthophyll-free basal diet. There was a significant difference in egg yolk color between each treatment, with the 1% treatment producing the closest to the ideal pigmentation. In trial 2, xanthophylls were extracted from the algae and added to the control ration in concentrations corresponding to those used in trial 1. The extract increased yolk color intensity, but not as much as corresponding concentrations of dried algae. Leghorn chickens

[1] Manuscript accepted July 1986.
[2] University of Hawaii, Department of Animal Sciences.
Sponsor: Dr. Ernest Ross.

were used in trial 3, where either dried spirulina or its extracted xanthophylls were added to both barley and corn basal rations. Again the dried algae had a slightly greater effect on yolk color than the corresponding level of extracted xanthophylls. Both pigment sources had a greater effect when added to the xanthophyll-free barley ration than when added to the corn basal diet. In trial 4, either untreated dried spirulina or algae from which xanthophylls had been extracted were used as protein sources for growing Japanese quail. No differences in growth rate or feed efficiency were observed between the control and treatments containing 2.5, 5, 10, or 20% of treated or untreated spirulina.

These studies demonstrated that xanthophylls could be extracted from spirulina and used as a supplementary pigment source for poultry diets. The residual algae could still be used as a protein feedstuff for poultry at up to 20% of the ration.

Growth in Gastropod Larvae: Benefits of a Natural Diet

JANICE L. BELL[3]

Growth rates of marine invertebrate larvae have a direct effect on the length of time spent in the plankton, and both can affect mortality in the plankton, timing of metamorphosis, proximity to recruitment sites, and success of recruitment. These in turn may be used to predict or explain success in terms of abundance, geographic distribution, or longevity in the fossil record. Actual growth rates of larvae in the plankton are difficult to determine. Thus rates of growth resulting from laboratory culture are generally considered the best estimates of larval growth.

The prosobranch gastropod *Crucibulum aculeata* was chosen for comparing growth rates in static laboratory cultures and those in natural seawater. Larvae were cultured in 0.45-μm Millipore filtered seawater in 1-liter glass beakers which stood partially submerged on a seawater table to maintain ambient temperatures. Larval density was 1 per milliliter or less. Larvae were fed monocultured phytoplankton in various combinations. Phytoplankton density in larval cultures was 1×10^4 cells per milliliter. Species included Tahitian *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Chaetoceras gracilis*, and a local flagellate. Larvae were also cultured in flow-through baskets made of 185-μm Nitex mesh and plastic beakers. To maintain circulation, an airlift constantly added drops of seawater to the top of the flow-through basket. Water entering the seawater table first passed into a settling bucket, which minimized sediment on the table.

Crucibulum aculeata hatched as a well-developed larva with a shell measuring 340 μm in length. In static culture, larvae grew fastest on a combination of Tahitian *I. galbana* and either *P. tricornutum* or *C. gracilis*, metamorphosing in 29 days after hatching. Larvae grown in flow-through seawater grew faster, however, reaching metamorphosis in 16 days. Metamorphosis was not induced by any specific agent or treatment. Metamorphic size ranged from 730 to 840 μm.

Particle counts showed coastal seawater to have $3–5 \times 10^3$ particles per milliliter in size ranges captured by larvae. Therefore the variety of food available in seawater may enhance growth in comparison with the diet of monocultured phytoplankton commonly used in laboratory culture. Thus conclusions drawn about a species, based on the length of larval life in culture, may need to be re-examined.

[3] University of Hawaii, Department of Zoology. Research supported in part by the Southwestern Malacological Society. Sponsor: Dr. Michael G. Hadfield.

Plant and Soil Water Status in *Marsilea villosa*

MARIE M. BRUEGMAN[4]

The tissue/water relations of *Marsilea villosa* were examined to determine the drought stress of this endemic Hawaiian fern under varying soil water potentials. Diurnal measurements of stipe water potential and leaf resistance were taken along with soil water potential. The Waller–Duncan k-ratio t-test was used to analyze the data. Plants growing in dry soil (< -0.10 MPa) had stipe water potentials and leaf resistances significantly different ($p < 0.05$) from plants in mesic and wet soils (0.0 to -0.10 MPa), but there was no significant difference between plants growing in wet (0.0 MPa) and mesic (-0.01 to -0.10 MPa) soils in greenhouse-grown plants. The results show that *M. villosa* is more drought-stressed under dry soil conditions. Plants under flooded conditions do not have significantly lower stipe water potential than mesic plants, indicating that drought stress does not occur due to waterlogging in flooded soils.

Pressure/volume curves were used to determine osmotic potential at full turgor. Results of the Duncan multiple-range test show that the osmotic potentials at full turgor of plants grown in dry and wet soils are significantly different ($p < 0.05$), and plants grown in soils of mesic water potentials are intermediate. These results are typical of plants growing in habitats where soil water potentials vary markedly through the growing season.

The Microbiological Quality of Cistern Water Supplies in Tantalus Used for Drinking and Bathing

ROBERT CHINN[5]

The Board of Water Supply does not provide water to residents living in the Tantalus area of Honolulu. Thus each household in this area must obtain its own water and usually uses a cistern system which collects rainwater from their roof to be stored in tanks. Although rainwater is of high purity, any contamination on the roof or gutters will be washed into the reservoir tanks. No agency is responsible for monitoring or ensuring the quality of these cistern water supplies. As a result, the quality of this cistern water which is used for drinking and bathing has not been determined. The objective of this study was to determine the microbiological quality of cistern waters in Tantalus based on current drinking water standards as established by EPA and the Hawaii State Dept. of Health. Eighteen households were included in this study population. Two water samples were collected from each household: one from the reservoir tank and the other from a household faucet. These water samples were analyzed for several water-quality indicator bacteria (total coliform, fecal coliform, fecal streptococci, total bacteria) by the membrane filtration technique as described in *Standard Methods*.

Current regulations dictate that good-quality drinking water should not contain

[4] University of Hawaii, Department of Botany. Sponsor: Dr. Charles H. Lamoureux.

[5] University of Hawaii, School of Public Health. Sponsor: Dr. Roger Fujioka.

more than four total coliform bacteria in a 100-ml water sample. Based on this criterion, 16/26 (62%) of the water samples obtained from the reservoir tank and 9/26 (35%) of the water samples obtained from household faucets exceeded the coliform standard for drinking water. The average concentration of total coliform for the 26 water samples was 72/100 ml and ranged from 0 to 1400/100 ml, while the average concentration of fecal coliform of these same samples was 116/100 ml and ranged from 0 to 1750/100 ml. Unexpectedly, more fecal coliform than total coliform bacteria were recovered from many of these samples. These results can be explained by the presence of high concentrations of total heterotrophic bacteria in these water samples which can interfere with the method to recover total coliform bacteria but not the method to recover fecal coliforms. Thus, analyzing cistern water for fecal coliform bacteria may be a more reliable test than analyzing these samples for total coliform bacteria as required by drinking water regulations. Based on these results, I conclude that many cistern water supplies in the Tantalus area do not meet the bacteriological drinking water standards and appear to be contaminated with fecal matter. The most likely sources of this fecal contamination are bird and rat droppings on the roofs and gutters of these houses. Exposing this water to sunlight may be one way of reducing the concentration of indicator bacteria.

The Use of Infaunal Invertebrates in Assessing the Effects of the Sand Island Sewage Outfall on the Marine Environment

DALE DAVIS[6]

Infaunal invertebrates associated with Honolulu's Sand Island deep-ocean sewage outfall were used to assess effects of sewage effluent on the marine environment. Replicate sediment samples were taken using a Shipek grab sampler at three stations (10, 70, and 140 m from the outfall to the west) and at a fourth site off Waikiki for the control. All infaunal invertebrates from these samples greater than 0.25 mm were identified, enumerated, and assessed for biomass. Methods used to analyze these data include species diversity, percent similarity, total abundance, and dominance. Results clearly show that infaunal communities at stations 1 and 2, at 10 and 70 m from the outfall respectively, are significantly different from the control. Total abundance, species diversity, and dominance values increase with increasing distance from the outfall, suggesting that there is a significant effect on the benthic community from the sewage outfall. Feeding guild analyses indicate that the abundance of filter feeders also increases significantly with increasing distance from the outfall. Grain size analysis, percent total organic carbon, and dissolved oxygen measurements do not show any significant differences between stations. Although it is apparent that the benthic community associated with the Sand Island sewage outfall is significantly affected by the sewage effluent, no cause-and-effect relationships have been established to explain the reasons for these changes. Results also indicate that any effects from the outfall are minor and localized to an area within approximately 70 m from the outfall.

[6] University of Hawaii, Department of Zoology. Sponsor: Dr. Julie Brock. This project was funded by the U.S. Environmental Protection Agency, contract CR811180-01-1, with Dr. S. V. Smith as principal investigator.

Growth, Reproduction, and Diet of *Myripristis amaenus* at Johnston Atoll

ANDY DEE[7]

Myripristis amaenus, the brick soldierfish or "menpachi," is a common inhabitant of coral reef communities and supports a sizable recreational and commercial fishery throughout the central and western Pacific including Hawaii. Little is known of the life history of this species or of most other members of the Myripristinae. Growth, reproduction, and diet were studied from collections of *M. amaenus* made at Johnston Atoll from February 1984 to January 1986. Otoliths were examined and growth increments counted using scanning electron microscopy to determine age and growth. Live specimens were marked using tetracycline and acetazolamide to validate the assumption of periodicity in layering of otolith increments. Growth increments appear to be formed daily. Size at first reproduction (SFR) was approximated by studying histological sections and by calculating and comparing the gonadosomatic index (GSI) for a range of mature and immature individuals from collections throughout the sampling period. The SFR suggested from GSI values for males and females is about 155 mm standard length. The GSI values indicate that major spawning occurs at least during the months of April and May, with some spawning also during January. *Myripristis amaenus* feeds primarily by night. Stomachs from specimens collected during the day yield few or no food items. The diet of *M. amaenus* consists primarily of crab megalops and alphaeid shrimps; smaller numbers of polychaetes and stomatopods are also taken. Gut contents of specimens collected around islands within the lagoon were similar to those of specimens collected elsewhere in the lagoon and in barrier reef habitats.

A Comparison of Hydrodynamic Forces on Two Sympatric Sea Urchins: Implications of Morphology and Habitat

WM. BRAD GALLIEN[8]

Fluid flow is a pervasive factor influencing the biology of intertidal organisms. Recent research has shown that the morphological design of many organisms limits the hydrodynamic forces they experience in rapid water flows, such as those accompanying wave break. One species of intertidal sea urchin, *Colobocentrotus atratus*, has a morphology which appears to be adapted for the high water velocities of its intertidal habitat. The aboral spines of this urchin species are reduced to tightly fitting, smooth plates, thereby reducing the cross-sectional area presented to the oncoming flow. The net result of this morphology is the reduction of hydrodynamic drag experienced by the urchin.

The force of drag that an organism experiences in moving water is proportional to its cross-sectional area, a coefficient related to its shape (the coefficient of drag) and the water velocity. Preliminary calculations based on an estimated coefficient of drag ($C_d = 0.5$, a typical value for hemispherical shapes) and water

[7] University of Hawaii, Department of Zoology. Sponsor: Dr. James D. Parrish. Funding source: Department of the Army, U.S. Army Engineer District, Honolulu, Fort Shafter, HI 96858, contract DACA83-84-C-0019; Hawaii Cooperative Fishery Research Unit, 2538 The Mall, UH, Honolulu, HI 96822.

[8] University of Hawaii, Department of Zoology. Sponsor: Dr. Stephen R. Palumbi. Funded through the help of a Sigma Xi Grant-in-Aid-of-Research.

velocities of 4 m/sec (typical for moderate wave heights) estimate the average force experienced by *Colobocentrotus* to be approximately 0.67 N/cm^2 of cross-sectional area, or 4 N for a 4-cm-diameter urchin. In contrast, the tenacity of the urchins, or their ability to cling to the rock, greatly exceeds this estimate of wave force. The breaking stress at which the urchin yields from the substratum is approximately 1 N/cm^2 of basal area, or roughly 10 N for the organism size used in estimating the 4-N drag force. Thus there seems to be a reasonable safety factor built into the design of this urchin. The same calculations for another intertidal urchin, *Echinometra mathaei* ($C_d = 1$, as measured for similar urchin species with the typical echinoid morphology), estimate their hydrodynamic drag to be 1.3 N/cm^2 of cross section under the same flow regime as above. The tenacity of *Echinometra* has not been estimated because of the rarity of encountering an individual foraging on exposed rocks, but even with the same tenacity as *Colobocentrotus*, the force of drag experienced by *Echinometra mathaei* would exceed its ability to cling to the substratum. Thus the streamlined morphology of *Colobocentrotus atratus* seems to allow the exploitation of a habitat where other organisms are excluded due to overwhelming hydrodynamic forces.

Age and Growth Estimation of the Pacific Blue Marlin, *Makaira nigricans* Lacepede, Using Otoliths, Vertebrae, and Dorsal and Anal Fin Spines

KEVIN T. HILL[9]

Billfishes are among the most important oceanic fishes today, as they are sought by both sport and commercial fishermen worldwide. Increased fishing effort and improved techniques in recent years have raised concern about the strength of billfish stocks. Japanese longline data for the Pacific blue marlin, *Makaira nigricans*, have been interpreted to indicate overfishing. Increased knowledge of vital life history aspects such as age and growth information is essential for sensible management of this fishery.

Age estimation of the Pacific blue marlin is still in the developmental stages, and although some data have been published using otoliths for this species, no other skeletal structures have been examined. The purpose of this study was to examine, interpret, and quantitatively compare growth patterns found in the otoliths, vertebrae, and dorsal and anal fin spines. Each hard part was evaluated for usefulness in terms of legibility and interpretability of growth patterns, ease of collection and processing, and the comparative precision of the resultant age data.

Blue marlin were sampled at various billfishing tournaments held in Kailua-Kona, Hawaii, during August 1982 and 1984. Skeletal hard parts and morphometric data were collected from 213 male and 104 female marlin. Individual round weight (kg), eye–fork length (EFL; cm), and sex were recorded. Males ranged from 95.4 to 222.0 cm EFL (19.1 to 138.8 kg), and females ranged from 125.7 to 398.8 cm EFL (20.9 to 748.0 kg).

Analyses indicated moderate to strong positive linear relationships between EFL and otolith weight, centrum cone depth, and spine width for most categories analyzed by sex. Otoliths, and both dorsal and anal fin spine sections, all contain growth patterns which were interpreted as annual events, and these events increase in number with the size of the fish. Vertebrae contain minute incremental growth marks which may represent some type

[9] University of Hawaii, Department of Zoology. Sponsors: Dr. Gregor M. Cailliet, Moss Landing Marine Laboratories, CA; and Dr. Richard L. Radtke, Hawaii Institute of Marine Biology. Research supported in part by the California Sea Grant College Program and the Packard Foundation.

of weekly or biweekly events, but no "annual" events were apparent. A paired *T*-test of age estimates revealed no significant difference in independent counts either within or between readers.

There was a direct linear relationship between age estimates of corresponding otoliths and dorsal and anal fin spines. Paired *T*-tests revealed no significant difference between age estimates of corresponding hard parts. There was a stronger relationship between corresponding dorsal and anal fin spine age estimates than between otoliths and these two hard parts.

Dorsal and anal fin spines proved to be more practical as aging material in terms of ease of collection, processing, legibility, and interpretation. Although they are more difficult to work with, otoliths may be more useful in terms of providing detailed age information from daily and other incremental patterns.

Mean length-at-age values were similar for otoliths and dorsal and anal fin spines. Males appear to grow to an average size of 176 cm EFL at 6 years, after which time their growth levels off rapidly. The largest male sampled (222.0 cm EFL) is the largest male on record, yet it was only estimated at 9 years. The oldest male was 18 years at 193.8 cm EFL. Growth of female marlin does not level off until a much later age than males. The largest female sampled (398.8 cm EFL) was also the oldest at 26 years.

Histological Evidence of Temporal Changes in the Ovaries of the Saddleback Wrasse *Thalassoma duperrey*

KAREN HOFFMAN[10]

Dynamics of ovarian development in teleost fishes often reveal patterns of reproductive readiness. Previous studies have suggested that spawning in the coral-reef fish, *Thalassoma duperrey*, may coincide with the daytime high tide. The present investigation seeks to address this possibility by quantifying temporal changes in ovarian components during the reproductive cycle. Although the gonadosomatic index (proportion of body weight devoted to gonad) has been commonly used to indicate reproductive state, microscopic examination of gonadal tissue sections reveals information about the components present in the ovary and the dynamics of these egg components over time. For this reason, standard histological procedures were employed to define egg stages and characterize daytime changes in egg development of the saddleback wrasse, *Thalassoma duperrey*, in fall and summer months of 1984 and 1985, respectively. Replicate fish were sampled hourly between sunrise and sunset in Kaneohe Bay, Hawaii. Time of catch was recorded in the field, and ovarian tissue was excised and fixed in Bouin's fixative in the laboratory.

Three distinct size classes (clutches) of oocytes were distinguished in the ovary based on microscopy. Clutches were segregated by oocyte diameter counts from transverse tissue slices of fixed ovarian tissue examined under a light microscope. Subsequent histological analyses (hematoxylin and Mallory stains) correlated size classes defined by the previous procedure to previtellogenic, vitellogenic (yolked), and hydrated egg stages. In the fall, previtellogenic and vitellogenic egg proportions show an inverse relationship during early hours; about half of the ovary consists of vitellogenic eggs and less than one-third is devoted to previtellogenic eggs. Both sharply change proportions around 2 hr prior to the daytime high tide. Hydrated egg proportions start to rise at this time; 1 hr later, they com-

[10] University of Hawaii, Department of Zoology. Sponsor: Dr. E. Gordon Grau.

prise one-third of the ovary. During the summer, previtellogenic and vitellogenic egg proportions are also inversely related; however, a previtellogenic group rather than a vitellogenic group dominates about half of the ovary in early hours. A decrease in vitellogenic eggs and concurrent increase in previtellogenic eggs occur around 2 hr prior to the high tide. Few or no hydrated eggs were observed in the summer. Smaller ovarian size as well as observed dominant previtellogenic egg proportions suggest reduced reproductive activities in the summer. In contrast, microscopic analyses reveal a greater incidence of hydrated egg stages and follicular remnants from ovulated eggs in fall ovarian samples, suggesting reproductively active gonadal tissue. This characterization of previtellogenic, vitellogenic, and hydrated egg stages and follicular remnants suggest greater reproduction in the fall. The dynamic changes in these features provide further evidence supporting the notion that reproduction coincides with the daytime high tide.

Butterflies and Butterflyfishes: Alternate Routes to Dietary Specialization?

THOMAS F. HOURIGAN[11]

Studies of dietary specialization in animals have concentrated on the specialization by insects such as butterflies on host plants. Theory predicts that specialized genotypes are favored by avoiding competition. Monophagy may evolve if an insect can utilize a plant species whose defenses make it unavailable to competitors. This theory predicts that such specialists should use resources which are less preferred by generalist species.

Evidence for this theory from studies on insects is contradictory, and there are few comparable studies of dietary specialization in other groups. Butterflyfishes which feed on corals present a potentially analogous situation to that of phytophagous insects. In this study, the feeding preferences of five species of Hawaiian corallivorous butterflyfishes were compared in the laboratory and in the field.

Two species of fishes, *Chaetodon quadrimaculatus* and *C. unimaculatus*, were specialists. Both fed on the coral *Pocillopora meandrina*, with *C. unimaculatus* feeding additionally on *Montipora* spp. These preferred coral species were not the most common corals in the habitat. The distribution of these fishes during 4 years of observation closely followed the distribution of the preferred corals. In laboratory experiments these fishes also preferred the same corals, often refusing to feed on other coral species.

The three remaining species, *C. multicinctus*, *C. ornatissimus*, and *C. trifasciatus*, had broader diets; most bites were directed toward *Porites lobata* and *P. compressa*, the most common species of corals. Nevertheless, all three species preferred *Pocillopora meandrina* and *Montipora* spp. to *Porites* spp. in the field as well as in the laboratory. These generalists occurred in all habitats, but their distributions more closely followed the distribution of preferred corals than the distribution of total coral cover. Laboratory tests revealed the same ranking of coral preferences as shown by the specialists, although some feeding was directed toward each coral.

Contrary to predictions of one theory explaining the evolution of monophagy in insects, specialists did not rely on corals which were less preferred by generalists. Instead, both preferred the same corals. Experiments suggest that the basis of these preferences may be that fishes ingest more energy per bite on preferred corals. If competition for preferred corals occurs, it may have consequences to the behavior of specialists.

[11] University of Hawaii, Department of Zoology. Sponsor: Dr. Ernst S. Reese.

Salt Transport Mechanisms in Brush-Border Membrane Vesicles of Tilapia Intestine

JAMES HOWARD[12]

Marine teleost fishes maintain their bodies at osmotic pressures that are approximately one-third that of seawater. In doing so they face a continuous diffusional gain of ions (predominantly sodium and chloride) and an osmotic loss of water. The strategy employed by these fishes for dealing with these adverse conditions is to drink seawater. The intestinal tract extracts 90–99% of the NaCl and 60–80% of the water from the ingested seawater. The salt is excreted across the gills while the water is retained. The only marine teleost in which intestinal transport mechanisms have been examined in detail is the winter flounder, *Pseudopleuronectes americanus*. Research on the winter flounder has provided evidence for a Na:K:2Cl symport entry mechanism for these salts across the brush-border membrane of intestinal epithelial cells. In mammalian epithelia both the Na:K:2Cl symport model and a Na–H/Cl–HCO_3 antiport model have been proposed for salt transport. The purpose of this study is to determine whether salt transport across the brush-border membrane of intestinal epithelial cells of *Oreochromis mossambicus* occurs via one, both, or neither of these two models.

Brush-border membrane vesicles (BBMV) from intestinal epithelial cells were isolated using a $MgCl_2$ precipitation/differential centrifugation technique. The uptake of Na or Cl into the vesicles was followed by placing the vesicles into solutions containing radioactive ^{22}Na or ^{36}Cl. At predetermined time intervals, aliquots of the reaction mixture were taken and passed through 0.65-micron filters. The radioactivity contained in the vesicles was measured using a scintillation counter.

Evidence to support the Na:K:2Cl model was not found for tilapia intestinal BBMV. The uptake of ^{22}Na into these vesicles was not stimulated by an inwardly directed KCl gradient. Evidence for support of the Na–H/Cl–HCO_3 model is not yet complete, but the presence of Na–H antiport has been disclosed. An outwardly directed pH gradient stimulated the uptake of ^{22}Na into the vesicles. Experiments to test for the existence of Cl–HCO_3 antiport are presently being conducted.

Results obtained so far indicate that a different mechanism may exist in tilapia (euryhaline) than in winter flounder (stenohaline) for transporting Na and Cl across the brush border of intestinal epithelial cells. This disparity may be due to the different salinity tolerances of these two fishes.

Changes in Growth Rate of Tilapia, *Oreochromis mossambicus*, Following Treatment with the Hormones Triiodothyronine (T3) and Testosterone

ROBERT D. HOWERTON[13]

Studies were undertaken at the Hawaii Institute of Marine Biology to assess the growth-promoting activity of testosterone (MT) and triiodothyronine (T3) in seawater-adapted tilapia. To determine the optimal dose of these hormones, a dose-specific growth response was characterized for both thyroid hormone and testosterone. T3 dose levels were 1, 5, 10,

[12] University of Hawaii, Department of Zoology. Sponsor: Dr. Gregory A. Ahearn. This work was supported by National Science Foundation grant PCM83-19973.

[13] HIMB/University of Hawaii, Department of Zoology. Sponsor: Dr. E. Gordon Grau. This research was made possible through funding by the University of Hawaii Sea Grant Program and the State of Hawaii Aquaculture Development Program.

and 25 mg/kg of feed. MT doses were 0.1, 1, 5, and 10 mg/kg of feed. Hormones were dissolved in 50 ml of ethanol and the solution sprayed on the surface of feed pellets. The control diet was sprayed with ethanol only. Diets were fed to treatment groups of 400 fish, at 4% of body weight, twice daily (8% total). Weight determinations were monitored biweekly for 10 months. At each weighing period ten fish from every treatment were randomly selected. These animals were sacrificed and blood samples taken to determine circulating thyroid hormone levels by radioimmunoassay. Condition and sex were recorded. Dramatic growth increases were evident in both thyroid hormone and testosterone treatments. Growth was inversely related to dosage in the thyroid treatment. The smallest dose (1 mg) resulted in the highest response, whereas the largest dose (25 mg) inhibited growth significantly. Growth and dosage were positively correlated in the MT treatment. All treatments resulted in increased growth rates; the highest dose (10 mg) was most effective. Fish from this treatment were more than twice the size of controls at the end of the treatment period. There was no significant difference in condition throughout treatment groups. Proximate analysis studies indicated that the hormone treatments produced no significant change in tissue water, protein, or lipid content. Sex ratio in the T3 treatment was similar to the control group whereas in the MT treatment the altered sex ratio was dose-dependent. The maximum dosage (10 mg) contained 94% males while the minimum dosage (0.1 mg) did not significantly alter the sex ratio.

Assessment of Genetic Diversity and Population Structure of the Reef Coral *Porites compressa* (Cnidaria, Scleractinia)

CYNTHIA L. HUNTER[14]

Methodologies previously used to identify genetic diversity in coral populations have been criticized as inadequate or ambiguous. Three independent assays were used to assess genetic diversity in clonal groups of the endemic Hawaiian coral *Porites compressa*. Colony morphology was described by measuring branch length and width, distance between branches, and corallum color. Tissue grafting was used as a bioassay of recognition of "self" (indicated by complete skeletal and coenenchyme fusion) or "nonself" (rejection responses). Electrophoretic analysis of five enzyme systems (glutamate dehydrogenase, malate dehydrogenase, phosphoglucomutase, phosphoglucose isomerase, and esterase) provided results in complete agreement with those of morphological and grafting data. No electrophoretic variation was detected among morphotypically identical or histocompatible colonies for the eight groups tested. A fourth technique was developed which measures individual variation in total composition and relative proportions of a suite of ultraviolet-absorbing compounds in coral tissue. Its application gave further corroborative evidence of genetic identity for 36 colonies in the eight groups assayed and provides a new method by which clonemates may be discerned.

Analysis of the numerical and spatial distributions of clonal groups (genetic individuals) defined by these four assays have shown that some genotypes (P3, P4, P5, P6, and P9) are common and collectively occupy more than 65% of the available substratum with 21, 16, 15, 31, and 38 colonies per 20 m^2, respectively, while others (P7, P8, P24) are relatively rare (7, 1, and 7 colonies per 20 m^2). "Common garden" experiments demonstrated that clonal dominance is related to relative growth rates, competitive ability, and the biotic and physical microenvironments of *Porites compressa* colonies. The overall fitness of each genotype is thus the additive result of the instantaneous fitness, or relative success in spatial occupancy, of each colony belonging to that particular clonal group.

[14] University of Hawaii, Department of Zoology. Sponsor: Dr. Robert A. Kinzie III.

Settlement Versus Recruitment in Sessile Marine Invertebrates

CATE HURLBUT[15]

Recruitment of sessile marine invertebrates as measured after a given period of time may not accurately assess settlement if significant juvenile mortality has occurred. Only when settlement is recorded after very short time periods can settlement be differentiated from postsettlement survival, or recruitment. I used daily photographs of acrylic settling panels to address the following questions: (1) Is settlement variable over time? Settlement on plates immersed for 24 hr (1-day plates) was compared between days. (2) Do previous recruits and/or microbial films affect settlement? Daily settlement was compared between 1-day plates and plates on which recruits accumulated for 14 days (14-day plates). (3) Does settlement equal recruitment over a 2-week period? If all settlers survive, then organisms present on day 14 will be equal to the sum of daily settlement.

The most abundant settlers were serpulids, the bryozoan *Schizoporella* sp., the bivalve *Anomia* sp., and the tunicates *Diplosoma* sp. and *Didemnum* sp. Settlement was variable over time on daily plates for *Schizoporella*, serpulids, and total tunicates. Daily settlement was significantly higher for all taxa on plates on which recruits accumulated as compared to 1-day plates, suggesting that microbial films or other recruits enhanced settlement. Mortality of juveniles was calculated for 14-day plates as the sum of daily settlement minus organisms present on day 14. This difference was significant for all groups. These results indicate that sampling of substrata 2 weeks after deployment underestimates settlement, as some juvenile mortality has already occurred. The need for a distinction between settlement and recruitment is thus emphasized.

Feeding Behavior of the Butterflyfish, *Chaetodon trifascialis*, at Johnston Atoll

DARBY IRONS[16]

The chevron butterflyfish (*Chaetodon trifascialis*) is found on coral reefs at Johnston Atoll and throughout the western Indo-Pacific. The current study examines the feeding behavior of this species at Johnston Atoll. This species is a territorial coral feeder strongly associated with *Acropora* spp. corals. Field observation data were recorded for 33 total individuals at three habitats in the lagoon. Habitat 1 had approximately 90% *Acropora cytherea* and approximately 5% *Montipora* spp. Habitat 2 had approximately 75% *Acropora cytherea* and approximately 15–20% *Montipora* spp. Habitat 3 had approximately 1% *Acropora cytherea* and approximately 90% *Montipora* spp. Feeding counts for each individual were conducted during ten consecutive 5-min periods. The number of bites taken on each coral species was recorded. The mean feeding rates differed widely between the habitats. This species had been previously reported to feed exclusively on *Acropora* spp. In the present study, individuals in a habitat with little *Acropora cytherea* feed occasionally on *Montipora* spp.

[15] University of Hawaii, Department of Zoology. Sponsor: Dr. Michael G. Hadfield.

[16] University of Hawaii, Department of Zoology. Sponsor: Dr. James D. Parrish. This research was funded by the U.S. Army.

The Dynamics of Hair Cell Production and Differentiation in the Inner Ear: DNA Labeling in the Embryonic Cochlea

AKIKO KATAYAMA[17]

In the chicken cochlea, hair cell phenotypes are not uniform; the size, the orientation, and the ultrastructure of the cilia arrays at the surfaces of hair cells vary in a manner that depends upon cell location in the sensory epithelium. Quantitative differences in some morphological features such as number, length, and width of the stereocilia vary systematically along the length and across the width of the sensory epithelium, suggesting that precise genetic and environmental control mechanisms regulate the manifestation of the cellular phenotypes. To learn what mechanisms control the differentiation of these phenotypes, it is essential to determine the pattern and the timing of hair cell production in this organ. Hair cell production was investigated in this study through autoradiographic localization of radioactively labeled DNA incorporated into the nuclei of newly produced cells.

Windows were made in egg shells and 25 μCi of ^{3}H-thymidine, a radioactive precursor of DNA, was injected into the yolk sac of chick embryos at various embryonic stages (embryonic day 3 through embryonic day 10). The eggs were resealed and incubated until embryonic day 11 when the embryos were sacrificed and their cochleae were fixed. The cochleae were sectioned and mounted on glass slides which were then coated with liquid NTB2 photographic emulsion and placed in light-tight boxes for 2 to 6 weeks. After development, dark silver grains of radioactively exposed photographic emulsion identified cells that contained DNA which had been replicated during the period when the ^{3}H-thymidine was available.

These experiments demonstrated that the majority of hair cells in the chick cochlea are produced after embryonic day 5. Preliminary data from animals injected on embryonic day 6 indicate that the first hair cells produced are those which lie in the center of the sensory epithelium. Along most of the cochlea's length, these initially produced cells form a band which runs longitudinally down the middle of the epithelium. Cells produced after embryonic day 6 form bands lateral to and paralleling the central band of initially produced cells. Hair cell production at the proximal end of the cochlea is almost complete by embryonic day 8 while cell divisions are still taking place at the lateral edges of the epithelium in the distal end of the cochlea. By embryonic day 10 the production of hair cells in the chicken cochlea is nearly completed.

The Microfauna Associated with Three Species of Coral and Their Accessibility to a Reef Community

ANN KITALONG[18]

Emergent microfauna were collected from a fringing reef adjacent to Coconut Island, Hawaii, from 24 May to 1 August 1983. Four substrates (sand, *Pocillopora damicornis*, *Porites compressa*, and *Montipora verrucosa*) were sampled from three zones (the lower,

[17] University of Hawaii, Department of Zoology/Bekesy Lab of Neurobiology. Sponsor: Dr. Jeffrey T. Corwin. This study was supported by a grant from NINCDS to Dr. Corwin.

[18] University of Hawaii, Department of Zoology. Sponsor: Dr. Julie Brock.

middle, and upper reef flat; the reef crest; and the reef front), using demersal traps (50 cm^2, 0.25 mm mesh). This study was conducted to determine whether emergent microfauna are associated with a given coral species or reef zone.

Most microfauna showed no significant association with a given coral species. Bursts of fish, crab, and shrimp larvae occurred in substantial numbers. These bursts were often sporadic "one-shot" occurrences. However, several species did show significant association with a given coral species. Large coral planulae of *P. damicornis*, associated with the adult, were collected in surprisingly great numbers throughout the study. Tunicate larvae showed an association with *M. verrucosa*, isopods were most abundant over *P. damicornis*, and *Acrocalanus inermis* were most abundant over *P. compressa*. Most microfauna showed no significant zonal preference. *Acrocalanus inermis* and isopods, predominantly "holoplanktonic" and epibenthic animals respectively, were ubiquitous throughout the reef. However, many holoplanktonic animals (cyclopoids, medusae, *Oikopleura diocia*, and tunicate larvae) were most abundant on the reef front. Several epibenthic animals (cumaceans, amphipods) were most abundant on the reef flat.

Recovery and Identification of Vibrio Bacteria from Commercial and Indigenous Oysters Placed in a Sewage-Polluted Estuary in Hawaii

CAROL J. KLING[19]

At least 14 species of vibrio bacteria have been reported to cause diseases ranging from gastroenteritis to external wound infections. This group of human pathogenic bacteria is unique in that their natural habitat is the marine water environment. Human infection occurs by the ingestion or handling of marine waters or marine foods. The objective of this study was to determine whether the nutrients in sewage effluent being discharged into an estuary would increase the concentrations of vibrio bacteria in the water and to investigate bioconcentration of the bacteria in seafood. The risk to human infection by the ingestion of marine foods was assessed by comparing the concentrations and kinds of vibrio bacteria recovered from the water column and from the tissues of a commercial oyster (*Crassostrea* sp.) and an indigenous oyster (*Isognomon* sp.).

The concentration of vibrio bacteria in two estuaries was compared. One estuary received no sewage effluent and was characterized by low phosphate (0.04 mg/liter) while the second estuary was characterized by high phosphate levels (1.37 mg/liter). Bacterial analysis of both water and oyster tissue was completed using the membrane filtration technique and selective media, TCBS (thiosulfate–citrate–bile salts–sucrose), for recovery and enumeration of vibrio bacteria. *Vibrio* species were confirmed by the API 20E (Analytab Products, Inc., Plainview, N.Y.) identification system. The concentrations of vibrio in the sewage-polluted estuary were ten times higher than in the unpolluted estuary and 167 times higher than in the open bay water. Concentrations of human pathogenic vibrio (*V. parahaemolyticus*, *V. vulnificus*) from oysters were 50 times greater than that recovered from the water column. These results indicate that there is a higher risk of vibrio infection from consuming oysters from estuaries in Hawaii that receive treated sewage effluent. This find-

[19] Hawaii Department of Public Health. Sponsor: Dr. Roger S. Fujioka. This research was funded by the Sea Grant College Program, University of Hawaii at Manoa.

ing is especially significant to the people in Hawaii because the water surrounding the Hawaiian Islands is warm (24 ± 2°C) throughout the year and is conducive to the growth of vibrio bacteria and because Hawaii has a high percentage of ethnic groups who eat many kinds of seafood with little or no cooking.

Allelopathy in Purple Nutsedge (*Cyperus rotundus* L.)

KEITH JEFFREY KOHL[20]

Yield loss in crops due to detrimental effects of weeds is a serious problem in agriculture. The interference may be caused not only by the competition for nutrients and water but also by the release of phytotoxic metabolites from the root systems of the weeds. The latter phenomenon is known as allelopathy. With the rising cost of herbicides and subsequent risks to our ecosystems, the allelopathic potential of crops and weeds may someday be used in integrated weed management programs to minimize the negative effects of weeds on crop growth while exploiting allelopathic interactions as potential pest controls.

Many previous reports indicating allelopathic activity in plant taxa have used root and tissue extracts or soil extracts of weed residues for isolation of phytotoxic compounds. In many cases where it was thought that root metabolites had been collected, in truth, damage to roots during collection may have been the main source of phytotoxic compounds found. Care should be taken, therefore, to assure that only root metabolites as they occur in the living plant are considered in allelopathic research.

Purple nutsedge (*Cyperus rotundus*), having vigorous growth and reproductive capabilities, has been termed "the world's worst weed" and affects crops in both tropical and subtropical regions. Phytotoxic compounds have been isolated from root/tuber extracts of *C. rotundus*, and residues in field studies have indicated their allelopathic potential. There is no evidence, however, that root metabolites of actively growing roots are responsible for observed phytotoxicity. This study examines the allelopathic potential of *C. rotundus*.

Nutrient solution in a circulating system was passed through a "donor" pot containing *C. rotundus* to a "receptor" pot containing germinating corn (*Zea mays*). A column containing either silica sand or Amberlite XAD-4 resin was placed between the donor and receptor pots. XAD-4 resin binds hydrophobic organic molecules and is a widely used adsorbent for studying trace organics in water. This continuous root exudate trapping system (CRETS) was constructed with chemically inert materials: purified water, glass pots, cleaned silica sand and basaltic rock, and glass tubing with Teflon connections to minimize organic contaminants. All glass parts of this system were covered with foil to prevent algal growth. Three CRETS containing sand columns and three containing XAD-4 resin columns were established. Columns were replaced every 2 days, and subsequent germination and growth of *Z. mays* was recorded for 19 days. Each receptor pot contained seven *Z. mays* seedlings.

Results showed that germination time was not significantly affected in CRETS with or without XAD-4 resin column. Subsequent growth, however, was greater in CRETS with XAD-4 resin columns. These results indicate that hydrophobic root metabolites of *C. rotundus* are inhibitory to *Z. mays* seedling growth. We are presently working to confirm this result and to test the susceptibility of other crops to *C. rotundus* root metabolites. We will also try to identify the phytotoxically active components of the metabolites and determine possible changes in their levels under water stress.

[20] University of Hawaii, Department of Botany. Sponsor: Dr. R. K. Nishimoto. This research is based upon work supported by the U.S. Department of Agriculture under Agreement 83-CRSR-2-2293.

Ventilation Rate as an Alternative Method of Measuring Metabolic Rate in *Macrobrachium rosenbergii*

DANETT DEE LI[21]

The use of the ventilation rate of *M. rosenbergii* was investigated as an alternative method to direct respirometry for measuring metabolic rate. The current procedure of monitoring oxygen consumption does not allow the metabolic rate of an individual to be measured while it is interacting within a group in a relatively natural situation. Experiments were undertaken to define the relationship between oxygen consumption and ventilation rate and establish the relation between ventilation rate and body weight.

A preliminary experiment measured ventilation rate by synchronizing a strobe light with the scaphognathite beat of isolated prawns in their holding aquaria. The coefficient of variation was determined for each prawn, and a conservative value was chosen to calculate the number of replicate measurements needed. A sample size of ten scaphognathite measurements per prawn was found to be adequate. The second experiment consisted of placing individual prawns into a respirometer, adapting them for 1 hr, and monitoring oxygen consumption over a 2-hr period. Scaphognathite rate was determined concurrently. There was a significant positive correlation between scaphognathite rate and oxygen consumption. During the final experiment a standard curve was developed between scaphognathite rate and both the wet and dry weights of prawns. Scaphognathite rate was measured for isolated animals in their holding aquaria with wet and dry weights subsequently determined. Significant negative correlations were obtained for scaphognathite rate with wet and dry weight. These results indicate that ventilation rate can be used as an alternative method for quantifying metabolic rate.

Modeling Nighttime Dissolved Oxygen Dynamics in Prawn Ponds of Hawaii

CHUCK MADENJIAN[22]

The ability to predict dissolved oxygen concentration (DO) in aquaculture ponds may serve as a valuable management tool, since it would furnish an advanced warning of low pond DO. Thus emergency aeration would be provided only when needed and energy expenses could be reduced. Dissolved oxygen, water temperature, and wind speed were monitored overnight at prawn *Macrobrachium rosenbergii* ponds of Amorient Aquafarm Inc. (Kahuku, Oahu) and at Brigham Young University–Hawaii prawn ponds (Laie, Oahu) from April through November 1985. A new model was developed to predict pond DO at dawn. The two components comprising the model included whole pond respiration and diffusion. Model inputs included a measurement of pond DO and water temperature at dusk, another DO and temperature measurement made several hours after the dusk measurement, and an estimate of the average wind speed for the night. For a lag of 3 hr between the dusk and second DO mea-

[21] University of Hawaii, Department of Animal Science. Sponsor: Dr. Lori B. Moore. Funding from the University of Hawaii Sea Grant College Program and State of Hawaii Aquaculture Development program is acknowledged and appreciated.

[22] Hawaii Institute of Marine Biology. Sponsor: Dr. Arlo W. Fast. This study was sponsored by grants from the University of Hawaii Sea Grant College Program (Project A/R-19) and the Hawaii State Aquaculture Development Program (Contract 17663) to Dr. Arlo Fast and Dr. Gary Rogers of the Hawaii Institue of Marine Biology.

surements, the mean absolute deviation of the predicted dawn DO from the observed dawn DO was 0.40 mg O_2/liter (for 40 verification trials). The model performed equally well for still nights (wind speed < 0.3 m/sec) and for relatively windy nights (wind speed near 10 m/sec). Contrary to conclusions of previous studies, diffusion of oxygen into the pond during the night was an important factor in determining dawn DO.

Spore Trek: Exploration of the Next Algal Generation

KARLA J. MCDERMID[23]

Ecological problems regarding space, herbivory, and life history multiplication among marine macroalgae are nearly the same as those that face sessile marine invertebrates. Information on marine invertebrate settlement and recruitment is increasing exponentially, but little is known about these processes in marine algae. The cooccurrence of nine or ten species of the red algal genus *Laurencia* in Hawaii made possible a comparison of early life history events in some of these species. The size of spores, their color, rate of germination, and early growth habits were found to vary among species. Reproductive effort fluctuates also, suggesting differences in spore output that would affect the numbers of the next generation. The perennial plants may change size through time, but they continue to occupy space that becomes limited for the young recruits. Apparently these *Laurencia* species produce mainly one kind of spore (tetraspores), which may imply a short-cycle life history, a strategy known in other algae for maintaining space.

Basolateral Glucose Transport in the Intestine of the Tilapia *Oreochromis mossambicus*

STEPHAN J. RESHKIN[24]

In mammals glucose transport from intestinal lumen to bloodstream has been shown to occur by two different transport systems on opposite sides of the intestinal epithelial cell layer. The intestinal brush-border membrane (facing lumen) of fish exhibits glucose transport properties similar to those found in mammalian intestine, except that fish have a lower absolute transport rate and different kinetics. Since transport rates and properties of fish basolateral membranes have not been characterized, our understanding of nutrient absorption in the most numerous vertebrate group is limited. This work describes the properties and role of the basolateral membrane in fish nutrient transport.

Characteristics of D-glucose transport by the intestinal basolateral membrane of the fish *Oreochromis mossambicus* were measured in isolated basolateral membrane vesicles (BLMV). Electron microscopy indicated that vesicles appeared to be generally closed with diameters in the range of 0.1 to 0.4 μm. The specific activity of the basolateral marker enzyme, Na,K-ATPase, was increased 11-fold, while specific activities of alkaline phosphatase and cytochrome *c* oxidase were enriched by a factor of 0.2 and 0.5, respectively. Transport was estimated by following the uptake of ^{3}H-D-glucose with the Millipore filtration technique.

Transport occurred by stereospecific facili-

[23] University of Hawaii, Department of Botany. Sponsor: Dr. Isabella A. Abbott.

[24] University of Hawaii, Department of Zoology. Sponsor: Dr. Gregory A. Ahearn. This work was supported by NSF Grant PCM83-19973.

tated diffusion and was not enhanced by either 120 mM gradients of NaCl or KCl. D-glucose transport was inhibited by sulfhydral reagents, phloretin, and cytochalasin B but not by phloridzin. Competition studies with a range of sugars demonstrated that aldohexoses in the C-1 chair conformation were the preferred substrates and probably share the same carrier. D-glucose (1 mM) initial influx, in the absence of cation gradients, was approximately 27 pmol/mg prot/sec. Kinetic analysis over a 200-fold glucose concentration range yielded a K_m for D-glucose transport of 11 mM and a V_{max} of 110 pmol/mg prot/sec.

This study demonstrates that the system for D-glucose transport in the basolateral membrane of fish intestinal epithelium closely resembles that found in mammal BLMV and in red blood cells. The values for initial transport rate and V_{max} are much lower than comparable rates found in mammals, while the value for K_m is only in the low end of the range found in mammals. This finding suggests that fish may maintain a slightly lower epithelial cell glucose concentration than mammals and may have a much lower rate of glucose transport into and out of the cell. Lower rates of fish brush-border glucose uptake and lower rates of fish metabolism lend support to this suggestion.

The Effects of Cadmium Exposures on the Immediate and Long-Term Growth Responses of Larvae of a Hawaiian Bivalve, *Isognomon californicum*

AMY HUFFMAN RINGWOOD[25]

Three-day-old *Isognomon californicum* larvae were exposed to four concentrations of cadmium (2, 10, 20, 50 μg/liter) to determine the effects of sublethal exposures on growth. The effects on growth were evaluated on the basis of shell dimensions (shell height and length) and dry weight for 28 days. Although size is commonly used to evaluate the effects of toxic substances on growth, weight was found to be a more sensitive indicator. Initially, a reduction in growth was observed, followed by a period of partial recovery during the second week of exposure. This partial recovery suggests that larvae possess mechanisms for sequestering or detoxifying cadmium. As Cd exposure continued, inhibition of growth prevailed. This pattern was also observed when larvae were 10 days old before Cd exposure was initiated. Therefore these responses are not an age-related phenomenon. The ability of larvae to recover when grown in clean seawater was also evaluated. After 14 days of Cd exposure, some of the larvae were transferred to clean seawater and grown for an additional 14 days. Recovery was observed only in the lowest Cd concentration.

Effects of Patch Reef Size and Isolation on Coral Reef Fish Recruitment

ROBERT E. SCHROEDER[26]

During the peak recruitment season (July–August 1984 and 1985) at Midway Lagoon, Northwestern Hawaiian Islands, experiments were conducted to determine whether either reef size or interreef isolation affects recruitment rate. Small, standardized attraction objects (coils of coarse wire mesh) served as artificial reef units. These attractors were arranged

[25] University of Hawaii, Department of Zoology. Sponsor: Dr. Michael G. Hadfield.

[26] University of Hawaii, Department of Zoology. Sponsor: Dr. James D. Parrish. Financial and logistic support were provided by the Hawaii Cooperative Fishery Research Unit, the University of Hawaii Sea Grant College Program, the Andrew J. Boehm Fellowship Award, Sigma Xi, and the 14th U.S. Coast Guard District.

on open sand flats at depths of 4 to 6 m. Newly recruited postlarval fishes were collected daily. The first design to analyze the effect of reef size on recruitment rate compared six adjacent attractors (simulating a large reef) to single-unit attractors. All attractors were isolated from other areas of hard bottom by about 100 m. The second design to analyze the effect of reef isolation compared a set of six attractors spaced 10 m from each other to single-unit attractors isolated by about 100 m. In general, daily total recruitment to the large "reef" was about four times greater than that to single-unit attractors. Daily total recruitment to the widely isolated attractors was higher than to the closely spaced units. These experimental results from the small artificial attractors were compared to population abundances of recently recruited fishes censused on much larger, neighboring, natural patch reefs that varied in size and isolation.

Evidence of Steroidogenesis in Postovulatory Follicles of the Tilapia *Oreochromis mossambicus*

CAROL J. SMITH[27]

Postovulatory follicles were examined with electron microscopy and enzyme histochemistry for sites of steroid hormone production. Light microscopy was also used to examine changes in the ovary with time after spawning. Electron microscopy detected the presence of smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubular cristae in certain cells of the theca interna. These structures are indicative of cells which manufacture steroid hormones. Granulosa cells also contained some smooth endoplasmic reticulum, along with an augmentation of Golgi complexes, vesicles, microvilli, and microfilaments within 5–7 days after spawning. Enzyme histochemistry demonstrated an intense reaction of 5,3β-hydroxysteroid dehydrogenase in variably placed thecal cells up to 7 days after spawning. At this time, the thecal cells of vitellogenic oocyte follicles also began to show strong 3-HSD activity. During the first 7 days after spawning, there was an increase in young primary oocytes and recruitment of some of these to vitellogenic oocytes. By 10 days after spawning, certain thecal cells in the follicle of these vitellogenic oocytes showed an intense 3-HSD reaction, while the postovulatory follicular tissue demonstrated a weak reaction. This arrangement continued for the life span of the postovulatory follicular tissue. Postovulatory follicles had a life span of up to 25 days after spawning in females that mouth-brooded. At 25 days after spawning, the postovulatory follicular tissue showed signs of degeneration with the presence of vacuoles and lysosomes. In females that did not mouth-brood, the postovulatory follicular tissue showed signs of degeneration at 10 days after spawning, and the next clutch of eggs developed at a faster rate than in females that did mouth-brood.

[27] University of Hawaii, Department of Zoology. Sponsor: Dr. Samuel R. Haley. This study was supported by an NSF Graduate Fellowship.

The Dispersal, Settlement, and Survivorship of the Larvae of a Colonial Ascidian as Observed in the Field Using SCUBA

DOUGLAS S. STONER[28]

The consequences of larval dispersal and habitat selection upon the distribution and abundance of the colonial ascidian *Diplosoma similis* were investigated by following the larvae of *Diplosoma* in the field using SCUBA. The study site was a leeward fringing reef located in southern Kaneohe Bay, Oahu. A total of 259 larvae were followed; of these, 122 successfully settled and metamorphosed.

Larval dispersal was predominantly confined to the source reef. Most of the 122 larvae settled within 4 min of release from the parent colony and dispersed 3 m or less. Almost without exception these larvae dispersed in the direction of the reef and settled either on the upper reef slope or the reef flat. Over 85% of the larvae settled on dead coral or the algae *Dictyosphaeria cavernosa*. Thirty larvae which swam up to the surface of the water and stayed there for an undetermined length of time may have dispersed to other reefs. However, because larvae are able to settle for at most an hour, successful dispersal between reefs is probably rare.

These data provide evidence that the abundance of *Diplosoma* is probably not limited by larval settlement rate since (1) larval abundance appears to be completely dependent on the size of the resident adult population, (2) the larval mortality rate was only 29%, and (3) reproduction in *Diplosoma* occurs year-round. However, the observed bias in the direction of larval dispersal toward the upper portions of the reef may partially explain the decrease in abundance of *Diplosoma* with depth. On the upper portions of the reef the abundance of *Diplosoma* is limited by heavy mortality experienced shortly after settlement. Over 50% of all juvenile colonies disappeared after 1 day postsettlement, and 98% had disappeared by the end of 2 weeks. The data further suggest that *Diplosoma*'s distribution is restricted to dead coral and *Dictyospaeria* as a consequence of larval habitat selection and not postsettlement processes.

Influence of Previous Crop and Inoculation with a Vesicular-Arbuscular Mycorrhizal Fungus on Mycorrhizal Infection and Growth of Sweet Potato

WHIDER SUN[29]

This experiment was conducted to determine the effect of previous crop and inoculation with a vesicular-arbuscular mycorrhizal (VAM) fungus on mycorrhizal formation and growth of sweet potato.

Chinese spinach (*Amaranthus gangeticus* L.), a nonmycorrhizal crop, and corn (*Zea mays* L.) cv. Hawaiian 68, a VAM host, were established at the Poamoho Experimental Station on 23 July 1985. The soil was a Tropeptic Eustrotox with medium phosphorus content (12.4 ppm Olsen P). Four plots of each species were replicated four times in a completely randomized block design. The experimental area was fumigated with methyl bromide to eliminate indigenous VAM fungi and soil-borne plant pathogens,

---

[28] University of Hawaii, Department of Zoology. Sponsor: Dr. John Stimson. This research was supported by a grant-in-aid from Sigma Xi and the Lerner-Grey Fund for Marine Research of the American Museum of Natural History.

[29] University of Hawaii, Department of Botany. Sponsor: Dr. Charles L. Murdoch.

especially nematodes. Both corn and Chinese spinach were inoculated with the VAM fungus (*Glomus fasciculatum* (Thax. sensu. Gerd.) Gerdemann and Trappe) Hawaii isolate 9. Nitrogen and potassium fertilizer was applied as needed.

After 90 days, the aboveground plant material was harvested. Two node-stem cuttings of sweet potato (*Ipomea batatas* L.) cv. Waimanalo were planted in the same plots 1 week later without soil cultivation. One-half of each plot was inoculated with *G. fasciculatum* Hawaii isolate 9 and the other half was uninoculated. Nitrogen and potassium fertilizer was applied as needed; however, no additional phosphorus was applied.

Growth rate of sweet potato was measured as the area of soil covered at 1, 2, and 3 months after planting. At 3 months, sweet potato tops were harvested. Dry weight and phosphorus content of tops were recorded, and VAM spore density was determined.

The number of VAM spores in plots following the VAM host corn was greater than in those following the nonmycorrhizal Chinese spinach. VAM spores in plots of uninoculated sweet potato following the nonmycorrhizal Chinese spinach may have been due to survival of resting spores of the original inoculation of Chinese spinach or to contamination from a surrounding plot.

The percentage of VAM infection of sweet potato roots was increased by inoculation in plots following Chinese spinach, but not in those following corn. Infection of inoculated sweet potato following Chinese spinach was not significantly different from that of inoculated or uninoculated sweet potato following corn.

VAM inoculation greatly increased ground cover of sweet potato following both crops at 2 and 3 months after planting. The magnitude of the increase was greater in plots following Chinese spinach. Ground cover of plots following corn was much greater than those following Chinese spinach, regardless of inoculation treatment.

Dry weight of tops of sweet potato following corn was greater than when following Chinese spinach, regardless of VAM inoculation. Dry weight of sweet potato was increased by VAM inoculation following the nonmycorrhizal Chinese spinach but not following corn.

The reduced growth of sweet potato following Chinese spinach is not satisfactorily explained on the basis of VAM suppression by a nonmycorrhizal crop. VAM infection of sweet potato which was inoculated with *G. fasciculatum* following Chinese spinach was not significantly different from inoculated or uninoculated corn. The area of ground covered at 2 and 3 months and the dry weight of tops, however, were much greater in plots following corn. These results indicate an allelopathic effect of Chinese spinach on the following crop of sweet potato which was not completely overcome by inoculation with a VAM fungus.

Orientation of Purified Basolateral Membrane Vesicles from Lobster Hepatopancreas Using Na^+, K^+-ATPase

ROBERT THOMAS TSUJI[30]

The biological significance of epithelial transport studies using isolated and purified luminal or antiluminal plasma membrane vesicles is dependent on orientation of the vesicles. Without establishing vesicle orientation—either inside-out (IO) or right-side-out (RO)—directionality of solute transport cannot be proposed for the cellular poles from which the vesicles came.

Basolateral membrane vesicles (BLMV)

[30] University of Hawaii, Department of Biochemistry and Biophysics. Sponsor: Dr. Gregory A. Ahearn. Funding was provided by NSF Grant DCB85-11272.

were purified from the hepatopancreas of Atlantic lobsters (*Homarus americanus*) using differential and step-gradient centrifugation. The orientation of BLMV was determined using ATPase activities in the presence and absence of a solubilizing agent (deoxycholate, DOC) and radioactive uptakes of ^{3}H-ouabain, ^{3}H-ATP, ^{3}H-alanine, ^{3}H-glucose, and ^{36}Cl.

There are theoretically three categories of vesicles in a basolateral membrane preparation: RO, IO, and leaky. RO vesicles are oriented with the ouabain binding site toward the external medium and the ATP binding site facing the internal medium. IO vesicles have the opposite orientation: the ouabain binding site faces the internal medium while the ATP binding site is exposed to the external medium. Both RO and IO are tightly sealed, impermeable to ATP and ouabain. Leaky vesicles have all ligand binding sites exposed simultaneously to the external medium and are permeable to ATP and ouabain. With its internalized ATP site, RO vesicles do not possess an ATPase activity. Because of the internal location of its ouabain site, IO vesicles only demonstrate a ouabain-insensitive ATPase activity. Because of their permeability characteristics leaky vesicles demonstrate a ouabain-sensitive ATPase activity. Using the inherent ATPase activities of the three categories of vesicles in the presence and absence of DOC, the percentage of leaky and IO vesicles can be determined. The remainder are RO vesicles.

Maximum stimulation of both total ATPase and Na^+, K^+-ATPase occurs at 0.05% DOC. This represents maximum opening of the tightly sealed vesicles exposing all ouabain and ATP ligand sites. The time courses of ^{3}H-ATP and ^{3}H-ouabain uptake are rapid, realizing 85–88% of their respective equilibrium values by 5 min of incubation. Total vesicular volumes, determined from the equilibrium values of ATP and ouabain, were 2.6 and 1.8 μl/mg protein, respectively. These values are significantly higher than the total vesicular volume per milligram protein (1 μl/mg protein) determined using ^{3}H-glucose, ^{3}H-alanine, and $^{36}Cl^-$ equilibrium values. The accumulation of ATP and ouabain, therefore, represent uptake into vesicles which are otherwise leaky to smaller molecules such as glucose, alanine, and Cl^-. Total vesicular volume is the sum of the two vesicle populations. Based on differential equilibrium volumes, 28–36% of all vesicles are sealed and 64–72% are leaky. These values are very close to those values determined by the enzymatic method: 62.9% leaky, 31.1% RO, and 6.0% IO. Both estimations suggest that the majority of vesicles are leaky to small solutes. The remaining third are tightly sealed, osmotically active vesicles. Of these the right-side-out orientation is favored over the inside-out orientation by about 5 : 1.

Sperm Predominance in Hawaiian *Drosophila*

ROBERT G. WISOTZKEY[31]

Sperm predominance was measured in multipli-mated female *Drosophila silvestris*. The three lab stocks used were Kilauea (U28T2), which is homokaryotic for a standard third chromosome, and Olaa (T94X) and Kahuku (U26B9), which are homokaryotic for the 3m inversion. Females were mated to males of one karyotype and then remated to males of the other karyotype. They were allowed to lay eggs for 6 weeks following the second mating. Paternity was determined by polytene analysis of salivary glands. Kilauea females had a significantly higher remating frequency than Olaa females (a remating frequency of 41% compared to that of 24%; $\chi^2 = 4.69$, $p < 0.05$). Kahuku females had a

[31] University of Hawaii, Department of Genetics. Sponsor: Dr. Hampton L. Carson. Research supported by NSF Grant BSR-8415633.

remating frequency of 34%, which is not significantly different from either of the other two.

The proportion of the larvae produced by the second male (P_2) was high (0.81); it was significantly different between Kilauea males and Olaa males. Means with 95% confidence intervals are 0.85 ($0.82 \leqslant \mu \leqslant 0.88$) and 0.74 ($0.67 \leqslant \mu \leqslant 0.81$) respectively. The P_2 of Kahuku males was not significantly different from the other stocks (0.79; $0.68 \leqslant \mu \leqslant 0.87$). The karyotype of the female did not appear to affect the P_2 of the male. There was no significant change in P_2 over the period following the second mating ($m = 0.002$, $t = 1.05$, $p < 0.05$), indicating that the sperm of the two males is mixed in the storage organs of the female.

Determination of the Relationship Between Larval and Juvenile Growth in *Macrobrachium rosenbergii* Using Genetically Marked Stock

DONIS T. ZACARIAS[32]

Larvae broods, genetically distinguishable from one another by their glutamate oxaloacetate transaminase (GOT) allozymes and hatched at different times, were combined into rearing tanks to simulate a larvae culture batch with more, and less, advanced stages ("early" and "late" hatchings). This procedure was carried out for larval batches designed to simulate single-parent (Part A) and multi-parent (Part B) larvae batches. In Part A the "intrafemale" broods were collected from two females who had synchronous hatching times: the early hatchings (with one GOT genotype) were collected from one female in the first half of the hatching period, and the late hatchings (having another GOT genotype) were collected in the last half of the hatching period. In Part B early hatches having one GOT genotype and late hatches having another GOT genotype were collected from several females with slightly asynchronous larval hatching times such that the early and late groups were greatly asynchronous with each other. Combining of early (E) and late (L) larvae from two GOT genotype options (for example: A, B) was carried out so that all relevant combinations were created (EA and LB, EB and LA, EA and LA, EB and LB, and so on). These larvae batches were reared and the relationship of the subadult population size classes were correlated with the genetically marked postlarvae metamorphosis and larvae hatching size classes. Preliminary results indicate that the slight initial size advantage that juvenile prawns have as a result of completing larvae to postlarvae metamorphosis more rapidly than others due to an earlier hatching time is magnified into large size differences in the subadult stages. Consequently we can conclude that much of the size variation in prawn populations is probably traceable to variable hatching times.

[32] University of Hawaii, Animal Sciences Department. Sponsor: Dr. Spencer Malecha. This study received support from the University of Hawaii Sea Grant College Program and the State of Hawaii Aquaculture Development Program.